

greater extent than to nucleic acids encoding other polypeptides, particularly other types of transporter molecules (or to complementary sequences thereof). The probe, which can contain at least 17 nucleotides (e.g., 18, 20, 25, 50, 100, 150, or 200 nucleotides) can be produced using any of several standard methods (see, e.g., Ausubel et al., "Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a GLUTX-specific nucleic acid sequence (for example, a nucleic acid encoding one of the transmembrane domains) that can be used as a probe to screen a nucleic acid library and thereby detect nucleic acid molecules (within the library) that hybridize to the probe.

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence that is the reverse and complement of the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the double helix). Complete complementarity between the hybridizing regions is not required in order for a duplex to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

Typically, hybridization conditions initially used to identify related genes are of low to moderate stringency.

These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high

conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

5 As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the lower the salt  
10 concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (e.g., room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be  
15 carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional  
20 parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will  
25 vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (e.g., the content  
30 of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (e.g., DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (e.g., on a

filter).

5 An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acid molecules are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

20 A second set of conditions that are considered "stringent conditions" are those in which hybridization is carried out at 50°C in Church buffer (7% SDS, 0.5% NaHPO<sub>4</sub>, 1 M EDTA, 1% BSA) and washing is carried out at 50°C in 2X SSC.

25 Preferably, nucleic acid molecules of the invention that are defined by their ability to hybridize with nucleic acid molecules having the sequence shown in SEQ ID NO:1 under stringent conditions will have additional features in common with GLUTX. For example, the nucleic acid molecules identified by hybridization may have a similar, or identical, expression profile as the GLUTX molecule described herein, or may encode a polypeptide having one or more of the biological activities possessed by GLUTX.

Once detected, the nucleic acid molecules can be